submitting an update to OTM to ask for reconsideration based on this new development.

Technology Summary – While embryonic stem cells can be maintained indefinitely in an undifferentiated state in culture, adult-derived stem cells typically do not display the same property of self-renewal. In fact, the biggest hurdle to the use of adult-derived stem cells is the difficulty in expanding the undifferentiated cell population *in vitro*. In particular, hematopoietic stem cells (HSCs) are rapidly depleted in culture – a recent report states it has "not yet been possible to identify a distinct hematopoietic stem cell with the capacity of self-renewal and in-vivo reconstitution of hematopoiesis" (*Cell Therapy: Technologies, Companies and Markets*, May 2004).

Dr. Cheng has shown that p18 deficient murine HSCs show dramatically improved self-renewal *in vitro* as compared with wild type HSCs. This is apparently accomplished not by increasing the rate of cell division, but by postponing differentiation of the cell. Not only were the HSCs able to maintain an undifferentiated state for a longer period of time in culture, they demonstrated a remarkable engraftment advantage, with a 14-fold greater abundance of long-term repopulating ability than wild type HSCs in irradiated mice. The dominance of the p18 deficient phenotype was observed in all major blood cell types, indicating multilineage differentiation, even in secondary recipients.

Recently, Dr. Cheng was able to demonstrate the same effect in human CD34 cells. He identified expression of the p18 protein in human HSCs and then used a targeted siRNA to reduce its expression. Using two different methods to deliver the siRNA to the cell he was able to produce p18 deficient HSCs and to maintain the cells *in*

vitro. He is currently examining the engraftment of these HSCs in NOD/SCID mice. Because of p18 expression is not unique to HSCs, it is anticipated that this approach could be used to enhance the *in vitro* expansion of other types of adult-derived stem cells as well.

Currently, the only known method of increasing HSC self-renewal is by genetically modifying the cell to express the exogenous gene, HOXB4. The current method provides advantages over the HOXB4 approach because it allows for transient disruption of p18 during *in vitro* expansion without the genetic manipulation of the cell. Such an approach is likely to be viewed more favorably from a regulatory perspective, as it minimizes the risk of unforeseen results from genetic manipulation, such as the development of leukemic transformation that was observed after transduction with HOXB8.

Requested Action – Under normal circumstances, OTM might reasonably wait for data from the SCID mouse experiments before making a decision to approve the current technology for patenting. However, because a provisional patent already exists, it will be necessary to take action prior to the filing deadline in October. If based on this new information, OTM decides that it wishes to approve the patent filing then the current data and the provisional would need to be combined in a full utility patent application. If OTM decides not to pursue patenting based on this information, Dr. Cheng requests that OTM release the new developments so that he may make arrangements for meeting the filing deadline on his own.

Ongoing studies in human hematopoietic stem cells:

To explore the potential applications of targeting p18 in human stem cell therapies, we have detected the expression of the p18 protein in human CD34 cells (Fig 11) and defined an effective small RNA interfering sequence for knocking the p18 protein down in the human cells (Fig 12). In addition, we have tried two approaches in delivering the siRNAs into human cells. One was the electroporation (Fig 13) and another one is lentiviral vector (Fig 14). With either method, we were able to achieve a considerable level of transduction in the human cells (more than 50%). A functional assessment after targeting p18 in human CD34 cells involving the use of NOD/SCID mice is currently under the way, (Fig 15).